

CLAIMS

1. A method for producing a molecular array which method comprises immobilizing to a solid phase a plurality of molecules at a density which allows individual immobilized molecules to be individually resolved, wherein each molecule in the array is spatially addressable and the identity of each molecule is known or determined prior to immobilization.
2. A method according to claim 1 wherein the molecules are applied to the solid phase by a method selected from printing, electronic addressing, in situ light-directed synthesis, ink jet synthesis or physical masking.
3. A method according to claim 2 wherein the molecules are applied to the solid phase by printing of dilute solutions.
4. A method for producing a molecular array which method comprises: (i) providing a molecular array comprising a plurality of molecules immobilized to a solid phase at a density such that individual immobilized molecules are not capable of being individually resolved; and (ii) reducing the density of functional immobilized molecules in the array such that remaining individual functional immobilized molecules are capable of being individually resolved; wherein each individual functional molecule in the resulting array is spatially addressable and the identity of each molecule is known or determined prior to the density reduction step.
5. A method according to claim 4 wherein the density of functional molecules is reduced by cleaving all or part of the molecules from the solid phase.
6. A method according to claim 4 wherein the density of functional molecules is reduced by functionally inactivating the molecules in situ.
7. A method according to claim 4 wherein the density of functional molecules is reduced by labeling some of the plurality of molecules such that individual immobilized labeled molecules are capable of being individually resolved.
8. A method according to claim 1 or 4 wherein the immobilized molecules are present within discrete spatially addressable elements.
9. A method according to claim 8 wherein the structure of molecules present in each discrete spatially addressable element is known and unintended structures are substantially absent.
10. A method according to claim 8 wherein a plurality of molecular species are present within one or more elements and each molecular species in an element can be distinguished from other molecular species in the element by means of a label.
11. A method according to claim 1 or 4 wherein the plurality of molecules which are capable of being individually resolved are capable of being resolved by optical means.
12. A method according to claim 1 or 4 wherein the plurality of molecules which are capable of being individually resolved are capable of being resolved by scanning probe microscopy.

13. A method according to claim 1 or 4 wherein the molecules are attached to the solid phase at a single defined point.
14. A method according to claim 1 or 4 wherein the molecules are attached to the solid phase at two or more points.
15. A method according to claim 1 or 4, wherein the molecules comprise a detectable label.
16. A method according to claim 15 wherein the label can be read by optical methods.
17. A method according to claim 15 wherein the label is a single fluorescent molecule, nanoparticle or nanorod, or a plurality of fluorescent molecules, nanoparticles or nanorods.
18. A method according to claim 15 where the label can be read by SPM.
19. A method according to claim 18 wherein the label is a non-fluorescent molecule, nanoparticle or nanorod.
20. A method according to claim 1 or 4 wherein the molecules are selected from defined chemical entities, oligonucleotides, polynucleotides, peptides, polypeptides, conjugated polymers, small organic molecules or analogues, mimetics or conjugates thereof.
21. A method according to claim 20 wherein the molecules are cDNAs and/or genomic DNA.
22. A method according to claim 1 or 4 wherein the immobilized molecules are present within discrete spatially addressable elements and each element comprises a distinct spatially addressable microelectrode or nanoelectrode.
23. A method according to claim 22 wherein said electrodes are formed of conducting polymers.
24. A method according to claim 23 wherein said electrodes are produced by a method selected from inkjet printing, soft lithography, nanoimprint lithography/lithographically induced self assembly, VLSI methods and electron beam writing.
25. A method according to claim 1 or 4 wherein the immobilized molecules are immobilized onto a single electrode.
26. A method according to claim 22 wherein the electrode (s) transduce a signal when a target molecule binds to an immobilized molecule present in the same element as an electrode.
27. A molecular array obtained by the method of claim 1 or 4.
28. A method for typing single nucleotide polymorphisms (SNPs) and mutations in nucleic acids, comprising the steps of : a) providing a repertoire of probes complementary to one or more nucleic acids present in a sample, which nucleic acids may possess one or more polymorphisms, said repertoire being presented such that molecules in said repertoire may be individually

resolved; b) exposing the sample to the repertoire and allowing nucleic acids present in the sample to hybridize to the probes at a desired stringency and optionally to be processed by enzymes; c) detecting individual reacted nucleic acid molecules after optionally eluting the unreacted nucleic acids from the repertoire.

29. A method according to claim 28, wherein the repertoire is arrayed on a solid phase.

30. A method according to claim 28, wherein said array is an array obtained by the method of claim 1 or 4.

31. A method according to claim 28, wherein the sample is exposed to a second repertoire of probes, which probes bind to one or more molecules of the sample at a different position to the probes of the first repertoire.

32. A method according to claim 31, wherein said first and second repertoires are differentially labeled.

33. A method for determining the complete or partial sequence of a target nucleic acid, comprising the steps of : a) providing a first set of probes complementary to one or more nucleic acids present in a sample, said first set of probes being presented such that arrayed molecules may be individually resolved; b) hybridizing a sample comprising a target nucleic acid to the first set of probes; c) hybridizing one or more further probes of defined sequence to the target nucleic acid ; and d) detecting the binding of individual further probes to the target nucleic acid. e) and detecting the approximate distance separating each probe or the order of each probe

34. A method according to claim 33, wherein the first set of probes is a repertoire of probes.

35. A method according to claim 33, wherein the repertoire is arrayed on a solid phase.

36. A method according to claim 35, wherein the target nucleic acids are captured to the solid phase at one or more points.

37. A method according to claim 33, wherein the repertoire is arrayed at a density which allows molecules in said repertoire to be individually resolved.

38. A method according to claim 33, wherein said array is constructed according to the method of claim 1 or 4.

39. A method according to claim 33, wherein the probes are differentially labeled.

40. A method for determining the number of sequence repeats in a sample of nucleic acid, comprising the steps of : a) providing one or more probes complementary to one or more nucleic acids present in a sample, which nucleic acids may possess one or more sequence repeats, said probes being complementary to a sequence flanking one end of the repeats, said probes being presented such that molecules may be individually resolved; b) contacting the nucleic acids with labeled probes complementary to units of said sequence repeats and a differentially labeled probe complementary to the flanking sequence at the other end of the targeted repeats; c) contacting the complex formed in b) with probes in a); and d) determining the number of repeats present on

each sample nucleic acid by individual assessment of the number of labels incorporated into each molecule and only counting those molecules to which the differentially labeled probe complementary to the flanking sequence is also associated with.

41. A method according to claim 40, wherein the repertoire is arrayed on a solid phase.
42. A method according to claim 40, wherein the repertoire is arrayed at a density which allows molecules in said repertoire to be individually resolved.
43. A method according to claim 40, wherein said array is constructed according to the method of claim 1 or 4.
44. A method for analyzing the expression of one or more genes in a sample, comprising the steps of : a) providing a repertoire of probes complementary to one or more nucleic acids present in a sample, said repertoire being presented such that molecules may be individually resolved; b) hybridizing a sample comprising said nucleic acids to the probes; and c) determining the nature and quantity of individual nucleic acid species present in the sample by counting single molecules which are hybridized to the probes.
45. A method according to claim 44, wherein the repertoire is arrayed on a solid phase.
46. A method according to claim 44, wherein the repertoire is arrayed at a density which allows molecules in said repertoire to be individually resolved.
47. A method according to claim 44, wherein said array is constructed according to the method of claim 1 or 4.
48. A method according to claim 44, wherein the repertoire comprises a plurality of probes of each given specificity.
49. A method for typing single nucleotide polymorphisms (SNPs) and mutations in nucleic acids, comprising the steps of : a) providing a repertoire of probes complementary to one or more nucleic acids present in a sample, which nucleic acids may possess one or more polymorphisms; b) arraying said repertoire such that each probe in the repertoire is resolvable individually; c) exposing the sample to the repertoire and allowing nucleic acids present in the sample to hybridize to the probes at a desired stringency and optionally be processed by enzymes such that hybridized/processed nucleic acid/probe pairs are detectable; d) eluting the unhybridised nucleic acids from the repertoire and detecting individual hybridized/processed nucleic acid/probe pairs; e) analyzing the signal derived from step (d) and computing the confidence in each detection event to generate a PASS table of high-confidence results; and f) displaying results from the PASS table to assign base calls and type polymorphisms present in the nucleic acid sample.
50. A method according to 49 wherein step (e) involves analyzing the signal from step (d) and computing in each detection event a FAIL table of low confidence results and using this table to inform primer and assay design.
51. A method according to claim 49 where the process is iterated for sequencing by synthesis.

52. A method according to claim 49, wherein confidence in each detection event is computed in accordance with Table 1.
53. A method according to claim 49, wherein detection events are generated by labeling the sample nucleic acids and/or the probe molecules, and imaging said labels on the array using a detector.
54. A method according to claim 28 or 49 where the SNPs that are probed are tags for a haplotype block or a region of linkage disequilibrium.
55. A method of obtaining allele frequencies by single molecule counting of pooled DNA.
56. A method according to claim 55 wherein obtained allele frequencies are used in association studies or other genetic methods.
57. A method according to 49 where probe and/or target acts as a primer or ligation substrate.
58. A method according to claim 49 wherein the probe and or target is enzymatically processed by ligases or polymerases or thermophilic varieties thereof or reengineered/shuffled varieties thereof.
59. A method according to claim 49 wherein the probe forms secondary structures which facilitate or stabilize hybridization or improve mismatch discrimination.
60. A method for determining the sequence of all or part of a target nucleic acid molecule which method comprises: (i) immobilizing the target molecule to a solid phase at two or more points such that the molecule is substantially horizontal with respect to the surface of the solid phase; (ii) straightening the target molecule, during or after immobilization; (iii) contacting the target molecule with a nucleic acid probe of known sequence; and (iv) determining the position within the target molecule to which the probe hybridizes.
61. A method according to claim 60 wherein the target molecule is contacted with a plurality of probes.
62. A method according to claim 60 wherein each probe is labeled with a different detectable label.
63. A method according to claim 60 wherein the target molecule is contacted sequentially with each of the plurality of probes.
64. A method according to claim 63 wherein each probe is removed from the target molecule prior to contacting the target molecule with a different probe.
65. A method according to claim 61 wherein the target molecule is contacted with all of the plurality of probes substantially simultaneously.
66. A method according to claim 64 wherein the probes are removed by heating, modifying the salt concentration or pH, or by applying an appropriately biased electric field.

67. A method according to any one of claim 28, 33, 40, 49, or 60 wherein the target is substantially a double stranded molecule and is probed by strand invasion using PNA or LNA.
68. A method according to claim 67 wherein the target nucleic acid molecule is a double-stranded molecule and is derived from a single-stranded nucleic acid molecule of interest by synthesizing a complementary strand to said single-stranded nucleic acid.
69. A method according to any one of claims 28, 33, 40, 49, or 60 wherein the target molecule is substantially single stranded and is made accessible to hybridization by elongation or stretching out.
70. A method according to any one of claims 28, 33, 40, 49, or 60 wherein a plurality of target molecules are analyzed simultaneously.
71. A method for determining the sequence of all or part of a target single-stranded nucleic acid molecule which method comprises: (i) immobilizing the target molecule to a solid phase at two or more points such that the molecule is substantially horizontal with respect to the surface of the solid phase; (ii) straightening the target molecule, during or after immobilization; (iii) contacting the target molecule with a plurality of nucleic acid probes of known sequence, each probes being labeled with a different detectable label; and (iv) ligating bound probes to form a complementary strand.
72. A method according to claim 71 wherein prior to step (iv), any gaps between bound probes are filled by polymerization primed by said bound probes.
73. A method according to claim 60 or 71 wherein the solid phase is a bead or particle.
74. A method according to claim 60 or 71 wherein the solid phase is a substantially flat surface.
75. A method for arraying a plurality of nucleic acid molecules which method comprises: (i) contacting the plurality of nucleic acid molecules with a plurality of probes, each probe being labeled with a tag which indicates uniquely the identity of the probe, such that each molecule can be identified uniquely by detecting the probes bound to the molecule and determining the identity of the corresponding tags; (ii) immobilizing the plurality of nucleic acid molecules randomly to a solid substrate; and optionally (iii) horizontalising and straightening the molecules, during or after immobilization.
76. A method according to claim 75 wherein the plurality of nucleic acid molecules are immobilized at a density such that individual immobilized molecules in the sample can be individually resolved.
77. A method according to claim 75 wherein the solid phase is a substantially flat solid substrate or a bead/particle/rod/bar.
78. An array produced by the method of claim 75.

79. A method for identifying and/or characterizing one or more molecules of a plurality of molecules present in a sample which method comprises: (i) producing a molecular array by a method comprising immobilizing to a solid phase a plurality of molecules present in a sample, wherein the plurality of molecules are immobilized at a density such that individual molecules in the sample can be individually resolved; and (ii) identifying and/or characterizing one or more molecule immobilized to the array by a method comprising contacting the immobilized molecules with a plurality of encoded probes; wherein each probe is encoded by virtue of being labeled with a tag which indicates uniquely the identity of the probe, such that an immobilized molecule can be identified uniquely by detecting the probes bound to the molecule and determining the identity of the corresponding tags.

80. A method according to claim 79 wherein the tagged probes are produced using combinatorial chemistry.

81. A method according to claim 79 wherein the tag is selected from a nanoparticle, a nanorod and a quantum dot.

82. A method according to claim 79 wherein each tag comprises multiple molecular species.

83. A method according to claim 79 wherein the tags are detectable by optical means.

84. A method according to claim 79 wherein the tags are particulate and comprise surface groups.

85. A method according to claim 79 wherein the tags are particulate and encase detectable entities.

86. A method according to claim 79 wherein tags can be detected and distinguished by scanning probe microscopy.

87. A method according to claim 79 wherein the solid substrate is selected from the group consisting of a bead, a particle, a rod and a bar.

88. A method according to claim 79 wherein the solid phase comprises channels or capillaries within which the molecules are immobilized.

89. A method according to claim 79 wherein the solid phase comprises a gel.

90. A biosensor comprising a molecular array obtained by the method of claim 1, 4, or 75.

91. An integrated biosensor comprising a molecular array according to claim 90 an excitation source, a detector, such as a CCD and, optionally, signal processing means.

92. A biosensor according to claim 90 wherein the biosensor comprises a plurality of elements, each element containing distinct molecules, such as probe sequences.

93. A biosensor according to claim 92 wherein each element is specific for the detection of a different target, such as different pathogenic organisms.

94. A biosensor according to claim 90 wherein the molecular array is formed on an optical fiber or waveguide.
95. A method according to claim 79 in which the plurality of probes are labeled with a tag which indicates uniquely the identity of the probe.
96. A method according to claim 28, 33, 40, 44, 49, 60, 71, 75, or 79 in which the plurality of tagged probes are hybridized substantially simultaneously or in groups of probes.
97. A method according to claim 28, 33, 40, 44, 49, 60, 71, 75, or 79 in which probes are grouped according to their T_m .
98. A method according to claim 79, in which each of the plurality of labeled probes are successively hybridized to the immobilized nucleic acid and a record of those that hybridize to each molecule can be used to identify or re-assemble the sequence of the immobilized molecule.
99. A method for determining haplotypes by probing single molecules immobilized on a solid phase in a spatially addressable manner.
100. A method according to claim 99 for haplotyping in which successive SNP sites are probed with different labels.
101. A method for haplotyping in which the first SNP is defined by the address of array element that binding occurs to and subsequent SNPs are defined by different labels.
102. A method for haplotyping on arrays, where first SNP is defined by address on array and subsequent SNPs are identified by solution probes.
103. A method for haplotyping on array captured and horizontalised and/or linearised DNA, where first SNP is defined by address on array and subsequent SNPs are identified by solution probes.
104. A method according to 103 where two different labels are used to distinguish members of the biallelic probe set and each successive SNP is identified by its position along the molecule.
105. A method according to 103 where errors are computed according to expected position of binding of probes along molecule.
106. A method where a population of molecules is analyzed and the haplotypes are computed according to the consensus of signals from single molecules.
107. A method according to claim 99, 101, 102, 103, or 106 in which haplotype frequencies can be determined.